1464

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS **88**, 94–97 (1960)

Influence of Magnesium Ion on the Ultraviolet Absorption of Aqueous Solutions of Salicylic Acid and Related Compounds

CHARLES A. ZITTLE, LEONARD PEPPER AND ELIZABETH W. BINGHAM

From the Eastern Regional Research Laboratory,[1] *Philadelphia, Pennsylvania*

Received September 30, 1959

The influence of magnesium ion on the ultraviolet absorption of salicylic acid and related compounds has been studied. Both salicylic acid and methyl salicylate are affected by the presence of magnesium ions. There is an increase in the absorption maximum, and the maximum shifts to longer wavelengths. This effect is marked only at higher pH values.

INTRODUCTION

In studies of the action of alkaline phosphatase on *O*-phosphorylsalicylic acid, it was observed (1) that magnesium chloride, used to obtain the full activity of the enzyme, increased the absorption of the unphosphorylated salicylic acid at 310 mμ at pH 10.0. These observations have been extended to include the effect of magnesium-ion concentration and pH on the absorption of salicylic acid at various wavelengths. The influence of magnesium ion on the absorption of a number of related compounds has also been studied.

MATERIALS AND METHODS

Maintenance of pH

The pH values were maintained by the use of 0.05 *M* buffers. Tris(hydroxymethyl)aminomethane-HCl buffer was used for the pH range of 7.0–8.0; ethanolamine-HCl buffer was used for the pH range of 9.0–10.0. Lower pH values were obtained by suitable adjustment with dilute HCl. In some experiments, dilute (0.01–0.04 *N*) HCl and NaOH served as the solvent medium.

Measurement of Ultraviolet Absorption

The light absorption was measured in the Beckman DU spectrophotometer, in a silica cell with a 1-cm. light path. Dilutions were prepared such that the maximum absorption was between 0.4 and 0.8.

RESULTS

Salicylic acid (*o*-Hydroxybenzoic Acid)

In Fig. 1 are shown the molar extinction curves for salicylic acid in 0.01 *N* HCl and in 0.05 *M* ethanolamine-HCl buffer, pH 10.0. Curves like the latter were obtained in buffers from pH 4.0 to 10.0, and also in 0.01 *N* NaOH. Doub and Vandenbelt (2) have observed that in 5 *N* NaOH, where the OH group of salicylic acid is ionized, the maximum molar extinction shifts to a wavelength of 306 mμ and has a value of 3400.[2]

In Fig. 2 is shown the effect of increasing concentrations of $MgCl_2$ on the absorption of salicylic acid at pH 10.0. The increase in the absorption of salicylic acid exerted by the presence of $MgCl_2$ is markedly influenced by pH. At pH 8.0 the increase by $MgCl_2$ is negligible, whereas between pH 9.5 and 10.0, there is a rapid increase to the values shown in Fig. 2. With higher concentrations of $MgCl_2$ at pH 10.0 and at higher pH values, precipitates, presumably $Mg(OH)_2$, prevent satisfactory measurements.

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture

[2] Dr. J. M. Vandenbelt has informed us that the OH group of salicylic acid is not completely ionized in 5 *N* NaOH, and that in 15 *N* NaOH, the maximum molar extinction shifts to a wavelength of 316 mμ and has the value 3400.

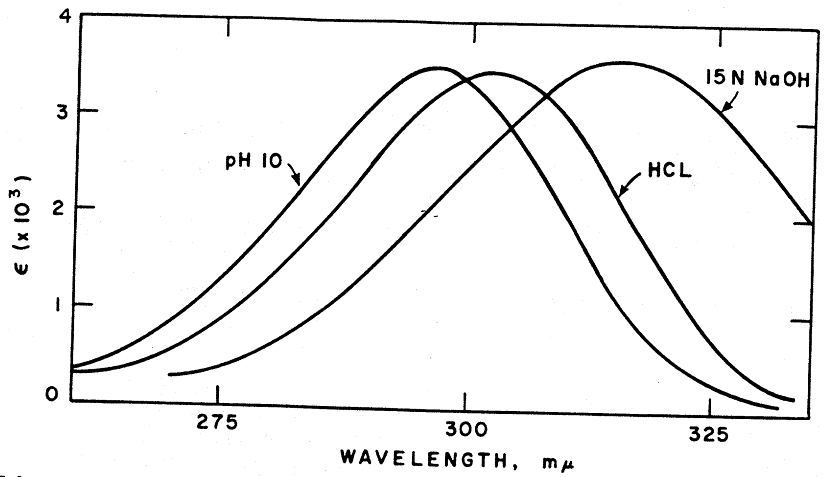

FIG. 1. Molar extinction coefficients (ε) for salicylic acid in 0.01 *N* HCl, and in 0.05 *M* ethanolamine-HCl buffer, pH 10.0, at wavelengths of 260–330 mμ. The data for the salicylic acid curve in 15 *N* NaOH were furnished by Dr. J. M. Vandenbelt.[2]

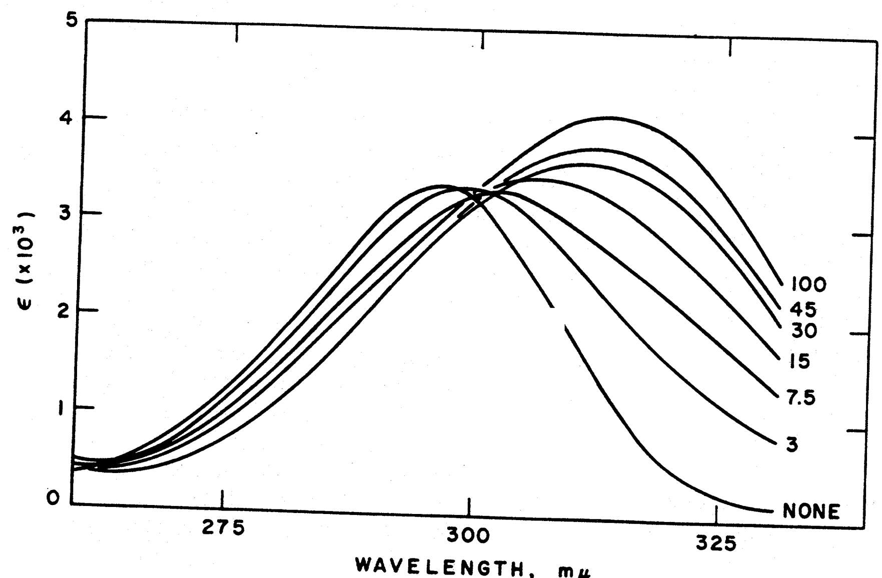

FIG. 2. Molar extinction coefficients (ε) of salicylic acid at pH 10.0, with various concentrations of $MgCl_2$, at wavelengths of 260–330 mμ. Numbers shown, divided by 1000, are the molar concentrations of $MgCl_2$.

Magnesium chloride was found to have a marked effect on the absorption of methyl salicylate also. The results for pH 10 are shown in Fig. 3. $MgCl_2$ at pH 8.1 had little influence on the absorption, whereas at higher pH values there is a successive marked increase to the values shown in Fig. 3. The light absorption of a number of related compounds (*O*-phosphorylsalicylic acid, phenol, benzoic acid, tyrosine, *p*-hydroxybenzoic acid, acetylsalicylic acid) were not affected by $MgCl_2$, or small ionic effects were observed that could be duplicated with NaCl.

DISCUSSION

The absorption of ultraviolet light by salicylic acid solutions has been studied in connection with the use of phosphorylsalicylic acid as a substrate for phosphatases (3, 4).

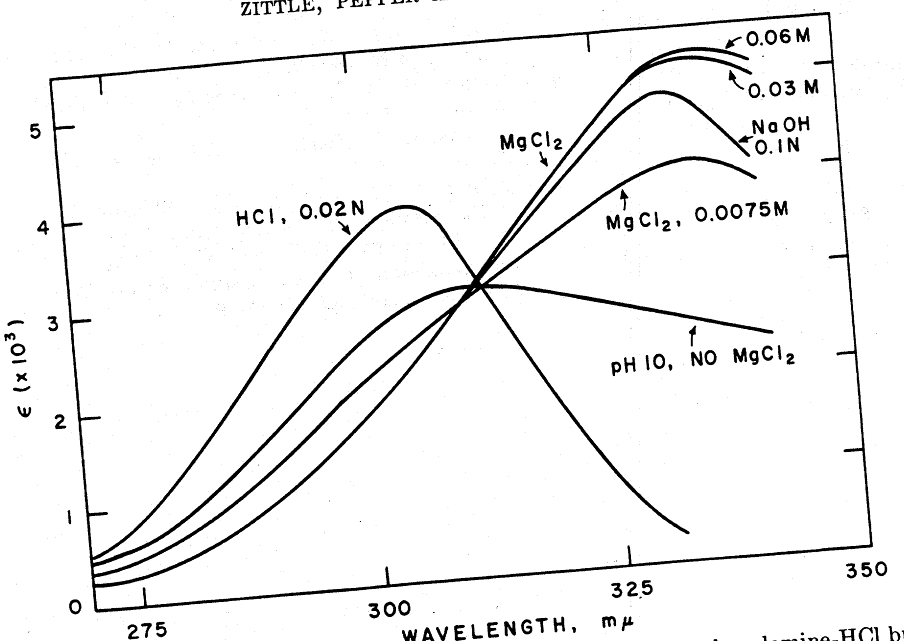

FIG. 3. Molar extinction coefficient (ε) for methyl salicylate in 0.05 M ethanolamine-HCl buffer, pH 10.0, with 0.0075, 0.03, and 0.06 M $MgCl_2$, at wavelengths of 270–340 mμ. Also shown are the absorption curves of methyl salicylate in 0.02 N HCl and in 0.10 N NaOH.

Hofstee (4) showed that the absorption at 310 mμ was constant from pH 10.0 to 4.0, and at lower pH values increased to a value about 60% greater at pH 1.0. The present studies show that this increase is due to a shift of the peak of the absorption curve from 296 to 302 mμ, and the maximum absorption at the peak changes very little. The present studies are in agreement with others (2, 5) that the absorption maximum of salicylic acid in acid is at 302 mμ with an ε value of 3600. With sufficient alkali to ionize the carboxyl group, the peak is at 296 mμ with an ε value of 3500. In 15 N NaOH,[2] in which the phenolic OH is ionized, the absorption peak is at 316 mμ with an ε value of 3400.

Of all the substituted benzene compounds examined for the effect of $MgCl_2$ on their ultraviolet absorption, only salicylic acid and methyl salicylate were affected, with a considerable shift in the position and the magnitude of the absorption peak. The structure of the salicylic acid at neutral pH values, with an ionized carboxyl group and a potentially ionizable phenolic hydroxyl group, suggested that the results with $MgCl_2$ were due to chelation. The influence of $MgCl_2$ on the absorption of methyl salicylate suggested that the negative charged carboxyl group was not specifically required for interaction. In this instance, it may be that the oxygen atom of the methyl salicylate carbonyl group provides a comparable site for interaction.

Changes in ionization and light absorption are observed with many chelations (6). Although there are examples of interaction between magnesium ion and chelating agent giving an absorption curve similar to that of the chelating agent in acid solution (6, 7), the interaction between $MgCl_2$ and salicylic acid or methyl salicylate appears not to be of this type. With these compounds, the change in absorption was in the direction of the fully ionized compound (phenolic OH) and not the neutral molecule. In both compounds there is good agreement between the position of the peaks in NaOH, where the phenolic OH is ionized, and at pH 10.0 with magnesium ion present. Thus in many respects the absorption curves obtained with $MgCl_2$ are comparable to the curves obtained when the phenolic OH groups of these compounds are ionized, an ionization that occurs normally at a higher pH value. (Tentative titration studies with salicylic acid suggest that the phenolic OH group dissociates about pH 10 in the presence of $MgCl_2$.)

REFERENCES

1. ZITTLE, C. A., AND BINGHAM, E. W., *Arch. Biochem. Biophys.* **86,** 25 (1960).
2. DOUB, L., AND VANDENBELT, J. M., *J. Am. Chem. Soc.* **71,** 2414 (1949).
3. BRANDENBERGER, H., AND HANSON, R., *Helv. Chim. Acta* **36,** 900 (1953).
4. HOFSTEE, B. H. J., *Arch. Biochem. Biophys.* **51,** 139 (1954).
5. EDWARDS, L. J., *Trans. Faraday Soc.* **46,** 723 (1950).
6. MARTELL, A. E., AND CALVIN, M., "Chemistry of the Metal Chelate Compounds," pp. 20, 101, 486. Prentice-Hall, New York, 1952.
7. SCHWARZENBACH, G., AND BIEDERMANN, W., *Helv. Chim. Acta* **31,** 678 (1949).